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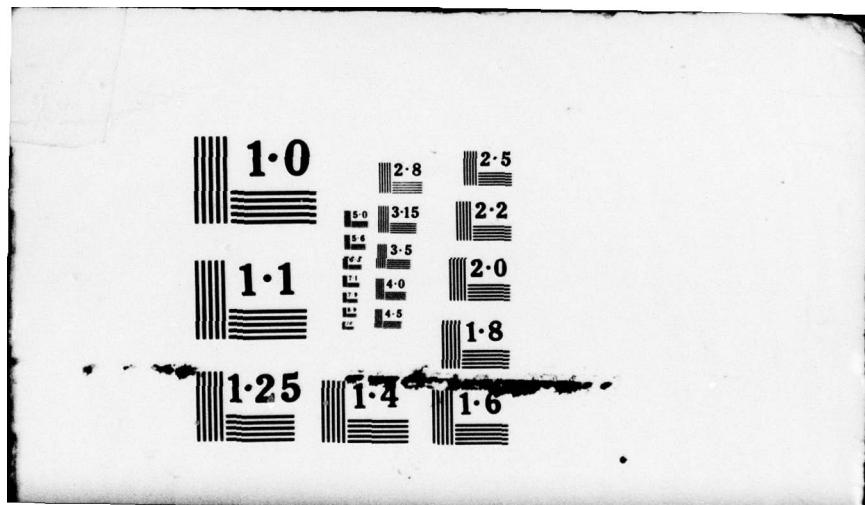
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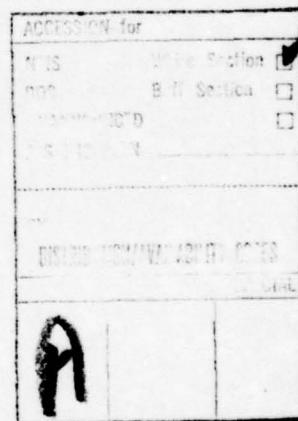
III. CONCENTRATIONS OF IMMUNOGLOBULINS IN DILANTIN INDUCED AND IDIOPATHIC GINGIVAL HYPERPLASTIC TISSUES

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INTRODUCTION

Diphenylhydantoin sodium (Dilantin), prescribed for extended periods of time for the treatment of epilepsy, frequently produces side effects and idiosyncratic reactions. Nystagmus, blurred vision, slurred speech, and ataxia are dose related side effects which usually subside following drug withdrawal.¹ Skin rashes accompanying manifestations of allergy to Dilantin have been reported in 5-10% of patients.²⁻⁴ Depression of one or more parameters of the cellular and/or humoral immune response has been described as occurring in 60%.⁵ Rarer reactions such as aplastic anemia, systemic lupus erythematosus, erythema multiforme,⁶ and Stevens-Johnson syndrome⁷ also occur. Fibrous hyperplasia of the gingiva occurs in 57% of patients on Dilantin therapy who are less than 26 years of age, but only in 21% of those who are older.⁸

There is general agreement that Dilantin therapy may cause IgA depression in 20-25% of patients, and that this drug-induced effect is reversible upon drug withdrawal.^{3, 9-13} Additional unknown factors may be required in addition to Dilantin to result in manifestation of IgA depression.¹⁴ Recent data suggest that epilepsy predisposes to low IgA, and IgA deficiency occurs only when hydantoins are administered.^{3, 14}

IgG and IgM levels in these patients appear to be much less consistently or significantly affected. Fontana et al.¹⁴ reported increased serum concentrations of IgG and IgM in epileptics irrespective of drug therapy when compared with blood donor controls. Slavin et al.¹¹ also found an elevation of serum IgG and IgM in 56 epileptics on anticonvulsant therapy. However, Seager et al.³ were unable to detect a change in serum IgG and IgM in 14

children on anticonvulsive therapy for epilepsy. In another study evaluating epileptics with and without Dilantin therapy,¹⁵ patients receiving Dilantin were found to have significantly lower serum IgG levels than controls, while IgM levels remained unchanged. Aarli¹³ found that decreases in IgG levels are more frequent during the first month of treatment, but are followed by a subsequent increase, and has suggested that these variations in immunoglobulin levels in individual epileptics may be related to the duration of treatment with Dilantin. Fossam¹⁶ reported that rabbits showed an altered immune response to antigens injected simultaneously with Dilantin. This altered response depended upon the nature of the antigen; the response to some antigens was suppressed, to some it was potentiated, and to some it was enhanced in the early phase and subsequently suppressed.

Because of the implication of Dilantin therapy in the alteration of the immune response, and the possibility that immune phenomena may function in the initiation and development of the periodontal diseases, including gingival hyperplasia, we compared the tissue levels of IgG, IgA, and IgM in normal, Dilantin hyperplastic, and idiopathic hyperplastic gingiva.

MATERIALS AND METHODS

Gingival tissue specimens were obtained from three groups of patients during routine periodontal therapy, consisting of a simple gingivectomy procedure. The surgical procedure was accomplished under local block and infiltration anesthesia using Xylocaine hydrochloride, 2%, with 1:100,000 epinephrine. The first group consisted of 15 patients (age 17-65 years; 12 males and 3 females) with clinically healthy gingiva (GI = 0, Löe and Silness¹⁷) requiring tissue reduction and recontouring following oral surgical procedures. Group II consisted of 11 patients (age 13-54 years; 9 males and 2 females) with clinically diagnosed gingival hyperplasia due to Dilantin

therapy. The minimum Dilantin dosage was 150 mg/day, the maximum 500 mg/day. Group III consisted of 8 patients (15-68 years; 4 males and 4 females) with idiopathic gingival hyperplasia.

Immediately following surgical removal, the tissue was rinsed in three changes of saline to remove surface blood, frozen within 30 minutes, and stored at -70C. Prior to homogenization the gingiva was thawed, diced into 1-2 mm cubes and thoroughly homogenized over ice in a 19x150 mm pyrex tissue grinder (No. 7725, Corning Glass Works, Corning, New York). Sufficient distilled water was added to the tissue to aid homogenization. The resulting white tissue homogenate was lyophilized and preweighed amounts (Cahn Electrobalance, Model 4100, Cerritos, Calif.) were reconstituted in TMED/acetate buffer pH 5,¹⁸ barbital buffer pH 8.6,¹⁹ and glycine buffer pH 10.3²⁰ for the quantitation of IgG, IgA, and IgM respectively. The specimens were assayed using low-level immunodiffusion plates (Behring Diagnostics, Somerville, N.J.) in accordance with the manufacturer's instructions except for the buffer modification and reference standard (World Health Organization serum standard, National Cancer Institute Immunodiagnostic Reference Center, Springfield, Virginia). In addition, electroimmunodiffusion assays as described by Bjerrum et al.,¹⁸ Weeke,¹⁹ and Grubb²⁰ were performed.

Assays for IgG, IgA, and IgM were performed on 31 gingival segments from the 11 patients with Dilantin hyperplasia. The majority of gingival specimens were obtained from the facial and lingual areas of the maxillary and mandibular anterior regions. The same immunoglobulin assays were carried out on 15 specimens obtained from the 8 patients with idiopathic

gingival hyperplasia. These gingival specimens were obtained from the facial and lingual areas of the anterior and posterior maxillary and mandibular regions. Normal gingival immunoglobulin values (previously reported,²¹ but included here for comparison) were determined by assay of clinically normal gingiva ($GI = 0$) from 15 patients. The immunoglobulin concentrations were expressed in μ g of immunoglobulin per mg of dry tissue. When more than one specimen for a patient was assayed, the results were averaged and the mean was utilized in the statistical analysis. Duncan's multiple range test,²² which is well suited for making contrast between all groups with balance between type I and type II errors, was used to analyze the RID (continuous) data. The normal approximation to the binomial²³ was used to test for differences in percentages of IgM.

RESULTS

Results of the radial immunodiffusion assays for the three groups of patients are shown in Tables 1-3 and summarized in Table 4. No significant differences in IgA levels between normal, Dilantin hyperplastic, and idiopathic hyperplastic gingiva were detectable. The highest and lowest IgA levels (patients 6 and 4, Table 1) were identified as outliers by Dixon's test²⁴ and were not used in the statistical analysis. Immunoelectrophoretic analysis of saliva and serum from patient 4 confirmed a complete IgA deficiency.

When compared with data for normal gingiva, a statistically significant increase in IgG was found in Dilantin hyperplastic tissue, but no significant increase could be shown for idiopathic gingival hyperplasia. The incidence of IgM detection was significantly higher in both Dilantin hyperplasia (90%)

and idiopathic gingival hyperplasia (75%) when compared to a 40% detection rate for normal gingiva.

The electroimmunodiffusion data will be published at a later date.

DISCUSSION

Dilantin induced gingival hyperplasia is of very complex etiology and pathogenesis. Various mechanisms postulated to induce Dilantin hyperplasia have included direct action by Dilantin on fibroblasts,^{25,26} a local response to metabolic products of Dilantin in saliva,²⁷ adreno-cortical derangement,²⁸ ascorbic acid deficiency,²⁹ and hypersensitivity.³⁰ None of these mechanisms have been fully substantiated.³¹ Recently it has been shown that Dilantin interferes with prolyl hydroxylase,^{32,33} an enzyme required for the posttranslational hydroxylation of prolyl residues in the synthesis of collagen.^{34,35} A reduction in prolyl hydroxylation has been associated with increased collagen production.³⁶

The possibility that Dilantin itself, directly or indirectly, may interfere with the normal degradation of gingival collagen, has attracted attention since hyperplasia may not necessarily be the result of increased fibroblastic activity; instead, it may be caused by an interference with collagenolysis during turnover, or the production of an altered collagen less easily degraded.³⁷ Evidence suggests that Dilantin affects connective tissue in other areas of the body as well as that in gingiva.³⁸⁻⁴⁰ The exaggerated effect on gingival connective tissue probably occurs because these tissues are constantly inflamed and susceptible to trauma.³⁷

The literature contains differences of opinion regarding the role of local irritants and inflammation in Dilantin hyperplasia. Although

Glickman and Lewitus⁴¹ have stated that improvement in local hygiene tends to reduce the inflammatory complications, but has little influence on the basic hyperplastic tendency, more recent evidence indicates that good oral hygiene and thereby minimal inflammation is essential in control and/or prevention of severe gingival enlargement.⁴²⁻⁴⁵ This concept is supported by animal studies wherein long-term Dilantin administration is effective in producing gingival hyperplasia only when accompanied by artificially induced plaque formation.^{30,45-48}

Clinically normal human gingiva harbors a minimal cellular infiltrate.⁴⁹ It is believed that plaque serves as a chronic antigenic stimulus for the numerous plasma cells⁵⁰ found in Dilantin hyperplastic gingiva.^{30,31,44,48,51,52} However, since abundant plasma cells are also present in nonspecific chronic gingival inflammation, they cannot be considered a characteristic feature. Consistent with the presence of plasma cells indicating inflammation and immune phenomena, particularly in altered humoral immunity, results of this study show a significant increase of IgG in Dilantin hyperplastic tissue. There was also a significant increase in the IgG level in Dilantin hyperplastic tissue compared to the IgG level of idiopathic hyperplastic tissue. However, no significant increase in tissue IgG levels was noted in idiopathic hyperplastic gingiva when compared to normal gingiva.

The significant elevation of IgG in Dilantin hyperplastic tissue may be due to the inflamed condition of the tissue as evidenced by the gingival index scores (Table 1). Gross et al.²¹ reported that in patients with moderate periodontitis, the mean IgG concentrations for inflamed gingiva with GI scores of 1 and 2 were 2.45 and 2.30 μ g/mg dry tissue, respectively.

These values approximate the mean of 2.92 $\mu\text{g}/\text{mg}$ for Dilantin hyperplastic tissue.

The differences in the mean IgG levels between idiopathic hyperplastic (1.78 $\mu\text{g}/\text{mg}$) and Dilantin hyperplastic tissues (2.92 $\mu\text{g}/\text{mg}$) may be related to the degree of inflammation indicated by the mean GI scores of 0.7 and 1.2 for the two groups (Tables 1 and 3).

IgM was detected in 90% of the Dilantin hyperplastic tissue specimens assayed and in 75% of the idiopathic hyperplastic tissue. This is in contrast to 40% for normal gingiva (GI = 0), 42% for mildly inflamed gingiva (GI = 1), and 47% for moderately inflamed gingiva (GI = 2).²¹

Genco et al.⁵³ has presented evidence supporting the concept that immune complexes are formed during periodontal disease. They suggest that plaque antigens entering gingival connective tissue will complex with antibody and be deposited in the tissue; or if they have affinity for a tissue component, will localize in that component. These immune complexes have been described as both enhancing⁵⁴ and blocking⁵⁵ local cellular immune reactions occurring in gingiva during periodontal disease.

Further studies are needed to determine if increased levels of IgG and IgM in Dilantin hyperplastic gingiva may be accompanied by an increase in immune complexes, particularly in light of the findings of Permin and Sestoft⁵⁶ who found deposits of IgG, IgA, and IgM at the dermoepidermal junction in the skin of all patients they examined who had been treated with Dilantin.

SUMMARY

Immunoglobulin levels in both Dilantin induced and idiopathic hyperplastic gingiva were determined and compared with the concentrations in normal gingival tissue. A statistically significant increase in IgG was found in Dilantin hyperplastic gingival tissue, but no significant difference could be shown for idiopathic gingival hyperplasia. IgA levels did not differ significantly in the three types of tissue assayed.

IgM was detected in 90% of the Dilantin hyperplastic gingival specimens assayed (mean GI = 1.2) and in 75% of the idiopathic hyperplastic tissue (mean GI = 0.7). This incidence of IgM detection was higher than that reported for normal and inflamed gingiva from periodontitis patients. The significance of these findings and possible correlation with the degree of tissue inflammation are discussed.

* * *

Commercial materials and equipment are identified in this report to specify the investigative procedures. Such identification does not imply recommendation or endorsement or that the materials and equipment are necessarily the best available for the purpose. Furthermore, the opinions expressed herein are those of the authors and are not to be construed as those of the U. S. Army Medical Department.

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TABLE 1. Immunoglobulin concentrations ($\mu\text{g}/\text{mg}$ dry tissue) in
Dilantin hyperplastic gingiva.

| <u>Patient</u> | <u>GI</u> | <u>IgA</u> | <u>IgG</u> | <u>IgM</u> ^ξ |
|----------------|-------------------|------------|------------|-------------------------|
| 4 | N.D. [§] | 0.00+* | 2.1 | N.D. |
| 6 | 2 | 2.15+* | 8.2 | 0.26 |
| 38 | N.D. [§] | 0.12 | 1.3 | 0.00 |
| 42 | 1 | 0.26 | 2.51 | 0.29 |
| 44 | 1 | 0.34 | 2.76 | 0.44 |
| 62 | 2 | 0.73 | 4.75 | 0.32 |
| 64 | 0 | 0.09 | 1.86 | 0.23 |
| 67 | 0 | 0.43 | 1.28 | 0.20 |
| 109 | 2 | 0.13 | 5.11 | 0.02 |
| 131 | 1 | 0.08 | 0.71 | 0.11 |
| 136 | 2 | 0.53 | 1.58 | 0.13 |
| Mean | 1.2 | 0.30 | 2.92† | 0.20 |
| Standard Error | | $\pm .08$ | $\pm .68$ | $\pm .04$ |

* Identified as outliers by Dixon's test and excluded from the statistical analysis.

† Statistically significant increase ($p < 0.05$).

ξ Significantly higher incidence of detection.

§ Not Determined

TABLE 2. Immunoglobulin concentrations ($\mu\text{g}/\text{mg}$ dry tissue) in
normal gingiva (GI = 0)

| <u>Patient</u> | <u>IgA</u> | <u>IgG</u> | <u>IgM</u> |
|----------------|------------|------------|------------|
| 59 | 0.14 | 1.01 | 0.23 |
| 72 | 0.69* | 6.84* | 0 |
| 91 | 0.03 | 0.68 | 0 |
| 92 | 0.05 | 0.39 | 0.23 |
| 49 | 0.78* | 4.29* | 0.39 |
| 110 | 0.11 | 1.42 | 0 |
| 119 | 0.05 | 0.58 | 0 |
| 125 | 0.11 | 0.82 | 0 |
| 118 | 0.07 | 0.76 | 0 |
| 120 | 0.05 | 0.70 | 0 |
| 115 | 0.25 | 1.90 | 0.16 |
| 128 | 0.15 | 1.06 | 0 |
| 138 | 0.09 | 0.26 | 0 |
| 151 | 0.02 | 0.58 | 0.04 |
| 153 | 0.11 | 0.81 | 0.04 |
| Mean | 0.095 | 0.84 | 0.07 |
| Standard Error | ± 0.02 | ± 0.72 | ± 0.03 |

*Identified as outliers by Dixon's test and excluded from the statistical analysis.

TABLE 3. Immunoglobulin concentrations ($\mu\text{g}/\text{mg}$ dry tissue) in idiopathic hyperplastic gingiva.

| <u>Patient</u> | <u>GI</u> | <u>IgA</u> | <u>IgG</u> | <u>IgM</u> † |
|----------------|-----------|------------|------------|--------------|
| 26 | 2 | 0.55 | 5.69* | 0.05 |
| 39 | 0 | 0.35 | 1.98 | 0.03 |
| 43 | 0 | 0.145 | 2.24 | 0.00 |
| 49 | 0 | 0.47 | 2.53 | 0.20 |
| 61 | 0 | 0.28 | 1.23 | 0.12 |
| 78 | 1 | 0.26 | 0.76 | 1.51 |
| 80 | 1 | 0.04 | 2.08 | 1.29 |
| 132 | 2 | 0.25 | 1.87 | 0.00 |
| Mean | 0.7 | 0.29 | 1.81 | 0.40 |
| Standard Error | | ± 0.06 | ± 0.23 | ± 0.22 |

* Identified as an outlier by Dixon's test and excluded from the statistical analysis.

† Significantly higher incidence of detection.

TABLE 4. Mean immunoglobulin concentrations ($\mu\text{g}/\text{mg}$ dry tissue) in gingiva.

| <u>Gingiva</u> | <u>IgA</u> | <u>IgG</u> | <u>IgM</u> |
|----------------------------|------------|------------|------------|
| Normal | 0.95 | 0.84 | 0.07 |
| Dilantin Hyperplastic | 0.30 | 2.92* | 0.20† |
| Idiopathic Hyperplastic | 0.29 | 1.81 | 0.40† |

* Statistically significant increase. ($P < 0.05$)

† Significantly higher incidence of detection.

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